METHODS FOR TREATING ENDOCRINE DISORDERS

by

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BACKGROUND

The present invention relates to methods for treating endocrine disorders. In particular, the present invention relates to methods for treating endocrine disorders by intracranial administration of a neurotoxin.

The pituitary gland (the hypophysis) is a relatively small weighing only about 600 mg. The pituitary can be divided into an anterior lobe (the adenohypophysis) and a posterior lobe (the neurohypophysis). The functions of both lobes of the pituitary are heavily influenced by vascular and/or neural connections with the hypothalamus. The anterior pituitary makes six major hormones and stores two others. These are growth hormone (GH) which regulates growth and has important functions on intermediary metabolism; prolactin (PRL) which is necessary for lactation and which can suppress menstruation; luteinizing hormone (LH) and follicle stimulating hormone (FSH) both of which influence gonadal activity in men and women; thyroid stimulating hormone (TSH) which regulates thyroid function, and; adrenocorticotropin (corticotropin (ACTH) which controls glucocorticoid function of the adrenal cortex. Vasopressin (AVP, also called antidiuretic hormone, ADH) and oxytocin are produced in neurons of the hypothalamus and stored in the posterior lobe of the pituitary. AVP controls water conservation by the kidneys, while oxytocin is necessary for milk let-down during lactation and may aid in parturition.

The hypothalamus weighs about 4 grams and is the ventral and medial region of the diencephalon forming the walls of the ventral half of the third ventricle. It is delineated from the thalamus by the hypothalamic sulcus (a shallow groove between the dentate gyrus and

5 the parahippocampal gyrus), lying medial to the internal capsule and subthalamus, continuous with the precommissural septum anteriorly and with the mesencephalic tegmentum and central gray substance posteriorly. The ventral surface of the hypothalamus can be identified by, proceeding from front to back along the ventral surface of the

10 hypothalamus, the optic chiasma the unpaired infundibulum which extends by way of the infundibular stalk into the posterior lobe of the hypophysis and the paired mamillary bodies. Hypothalamus neurons have been grouped into various nuclei on the basis of functional characteristics. These hypothalamic nuclei include the supraoptic

15 paraventricular, lateral preoptic, lateral hypothalamic, tuberal, anterior hypothalamic, ventromedial, dorsomedial, arcuate, posterior hypothalamic, and premamillary nuclei and the mamillary body.

The hypothalamus has afferent nerve fiber connections with the

20 mesencephalon, limbic system, cerebellum and efferent fiber connections with the same structures and with the posterior lobe of hypophysis. Functional connection of the hypothalamus with the anterior lobe of the hypophysis is established by the hypothalmo- hypophysial portal system. The hypothalamus is prominently involved in

25 the functions of the autonomic nervous system and, through its vascular link with the anterior lobe of the hypophysis, in endocrine mechanisms. The hypothalamus may also play a role in neural mechanisms underlying moods and motivational states. With regard to the endocrine hypothalamus, it is known that the arcuate nucleus of the hypothalamus

30 is the major site of gonadotropin hormone releasing hormone (GnRH) production.

A single hypothalamic regulatory factor, GnRH, stimulates synthesis and secretion from the anterior pituitary of both FSH and LH in males and females. FSH and LH are collectively referred to as the gonadotropins. Other hypothalamic regulatory factors stimulate pituitary

5   secretion of ACTH, TSH and GH, but inhibit the release of PRL from the anterior pituitary.

Vascular connection of the hypothalamus to the hypophysis is established when the neurosecretory neurons of the hypothalamus

10   release regulatory (releasing and inhibiting) factors which reach the adenohypophysis through the portal circulation of the capillary plexus located in the median eminence region of the hypothalamus. These neurohemoral hypothalamic regulatory factors are small peptide neurohormones which, by influencing the secretion of adenohypophysial

15   hormones, serve as the main functional link between the brain and the adenohypohysis. The hypothalamus is itself influenced by higher brain regions which employ various neurotransmitters, including dopamine, norepinephine, serotonin and acetylcholine to act upon the hypothalamus.

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Addition of acetylcholine to anterior pituitary tissue incubated with hypothalamic fragments can result in the release of LH by the pituitary tissue and this LH release can be blocked by atropine (a muscarinic acetylcholine receptor (post synaptic) antagonist). Prostigmine, an

25   acetylcholinesterase inhibitor, can also stimulate LH release in this system. Fiorindo R.P. et al., *Evidence for a Cholinergic Component in the Neuroendocrine Control of Luteinizing Hormone Secretion*, Neuroendocrinology 18: 322-332, 1975.

30   Additionally, while as expected, FSH levels increased upon addition of hypothalamic fragments to incubated anterior pituitary tissue, a further increase of pituitary FSH release is produced by acetylcholine. This

enhanced FSH release is antagonized by atropine. Simonovic I, et al., *Acetylcholine and the Release of Follicle Stimulating Hormone Releasing Factor,* Endocrinology 95: 1371,1974.

5   Furthermore, cholinergic agents can stimulate release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which is blocked by atropine. GnRH acts in turn upon the anterior pituitary to promote the release of LH and FSH. Richardson SB, et al., *Acetylcholine, Melatonin, and Potassium Depolarization Stimulate*

10   *Release of Luteinizing Hormone-Releasing Hormone From Rat Hypothalamus In Vitro,* Proc Natl Acad Aci U.S.A. 79 (8): 2686-9,1982. In intact rats, injection of acetylcholine into the lateral ventricle stimulates LH release. This can be blocked with atropine or enhanced with prostigmine.

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Notably, hemicastration (removal of one ovary) in the female rat normally results in gonadotropin-mediated hypertrophy of the remaining ovary and this effect be blocked by atropine implants into the anterior hypothalamus. Monti J.M. et al., *Inhibition of Ovarian Compensatory*

20   *Hypertrophy by Implants of Atropine in the Hypothalamus,* Experientia 26: 1263-1264,1970. Similar implants also blocked the post-orchidectomy rise in LH and FSH in male rats. Libertun C. et al., *Blockade of the Postorchidectomy Increase in Gonadotropin by Implants of Atropine into the Hypothalamus,* Proc Soc Exp Biol Med 152: 143-

25   146,1976. These data are consistent with the existence of a muscarinic cholinergic mechanism mediating GnRH release in the hypothalamus, clearly indicating therefore a stimulatory cholinergic influence over hypothalamic activity.

30   Additional studies have indicated that the net effect of acetylcholinesterase inhibition or acetylcholine administration to the hypothalamus stimulates gonadotropin secretion. See e.g. Blake C.A.,

Parallelism and Divergence in Luteinizing and Follicle Stimulating Hormone Release in Nicotine-Treated Rats, Proc Soc Exp Biol Med 145: 716-720, 1974; Kanematsu S. et al., *Inhibition of the Progesterone-Advanced LH Surge at Proestrus,* Proc Soc Exp Biol Med 143: 1183-

5   5,1973; Blake C.A., et al., *Nicotine Delays the Ovulatory Surge of Luteinizing Hormone in the Rat,* Proc Soc Exp Biol Med 141: 1014-1016,1972; Blake C.A. et al., *Localization of the Inhibitory Actions of Estrogen and Nicotine on Release of Luteinizing Hormone in Rats,* Neuroendocrinology 16: 22-35,1974, and; Blake C.A. et al., *Effects of*

10   *Hypothalamic Deafferentation and Ovarian Steroids on Pituitary Responsiveness to LH-RH in Female Rats.* In Gual and Rosemberg, eds, Hypothalamic Hypophysiotropic Hormones. Excerpta Medica, Amsterdam, 1973, pp. 33-38.

15   With few exceptions (testosterone in men, progesterone in men and women) hormone excess causes pathological effects. Thus, excessive secretion of growth hormone or GHRH causes acromegaly in adults or gigantism in adolescents, if the excessive secretion starts before epiphyseal closure. With regard to prolactin, pathological

20   hyperprolactinemia is a common cause of infertility in women, since elevated prolactin level results in suppression of a normal menstrual cycle. The primary physiological effect of the gonadotropins is the promotion of gametogenesis and/or gonadal steroid production. In the male, LH stimulates production of testosterone. Testosterone is

25   required for sperm production, maintenance of sexual libido and secondary sexual characteristics. FSH is required for normal sperm production. In the female, the overall effect of FSH is to promote the growth of the developing follicle, whereas the overall effect of LH is to induce ovulation.

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Current therapies for endocrine hypothalamic and pituitary disorders have significant drawbacks and deficiencies. Treatment for endocrine

disorders primary to pituitary hyperplasia include medical (drug) therapy. Thus, prolactin-secreting pituitary adenoma (prolactinoma) has been treated with systemically administered bromocriptine or other dopamine agonists. Unfortunately, tumor activity typically returns when drugs are

5 withdrawn. When medical therapy is unsuccessful, surgical resection, by transphenoidal surgery, is a standard procedure. The pituitary gland can be surgically approached by the transphenoidal (through the sphenoid sinus to the pituitary fossa), transethmoidal (through an incision in the medial orbital wall), transoral and transfrontal (craniotomy)

10 routes. See e.g. Lindsay K.W. et al., *Neurology and Neurosurgery Illustrated*, pages 303 and 332, Churchill Livingstone, third edition (1997). Surgical intervention carries the risk of incurring a dysfunction induced by the surgery itself, such as hypopituitarism (including diabetes insipidus) and visual loss, as well as mortality.

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Radiosurgery, including stereotactic radiosurgery has been used to treat various hyperplasic and hypertrophic pituitary disorders, including pituitary neoplasms. See e.g. Williams, J. et. al. *Stereotactic Radiosurgery for Cushings Disease and Prolactinoma*, Journal of

20 Radiosurgery Vol. 2, No. 1, pages 23 - 29, 1999. Radiation therapy carries the risk of hypopituitarism and radiation injury to the brain and optic apparatus.

The human hypothalamus was practically surgically inaccessible until

25 the advent of stereotactic surgery and the earliest reported stereotactic surgery performed upon a human hypothalamus was by Speigel and Wyeis in 1949. Stereotactic neurosurgery has been practised for therapeutic hypothalamotomy through use of chronic electrical stimulation or permanent ablative lesioning for effective palliation of

30 functional aberrations of the hypothalamus to treat various indications, including erethistic hyperkinesis, intractable pain, addiction (alcohol and drug) and sexual hyperkinesis. See e.g. Sharma, R.R., et al., *Minimally*

Invasive Neurosurgery Using CRW-3 Stereotaxy, Ann Saudi Med 1994;14(6):507-510. Hypothalamic stereotactic target coordinates have included from 1 mm anterior to the midpoint of the intercommisural line to 2 mm posterior to the midpoint, 2 to 4 mm below the intercommisural

5 line, and 2 mm lateral to the lateral wall of the third ventricle. Notably, stereotactic therapeutic lesioning hypothalamotomy has been carried out by injecting wax through a stereotatically inserted needle. Hunter, S., *Stereotactic Hypothalamotomy*, chapter 153, pages 1507-1517 of Textbook of Stereotactic and Functional Neurosurgery, edited by

10 Gildenberg P.L. et al., McGraw-Hill (1998). A device useful for chronic intracranial delivery of a pharmaceutical is disclosed by PCT/US99/17880 (WO 00/07652).

Stereotactic techniques developed initially for lesion making, enable

15 accurate placement of a cannula or electrode at a predetermined target site within the brain. Many different stereotactic frames have been developed, e.g. Leksell, Todd-Wells, Guiot. These combined with radiological landmarks (usually ventriculography or use of CT/MRI) and a brain atlas permit anatomical localization to within about one mm.

20 Since some functional variability occurs at each anatomical site, localization can also based be upon recorded neuronal activity and the effects of electrical stimulation. Stereotactic surgery permits millimeter precision placement of a probe, electrode, catheter, or hollow needle at a desired intracranial location. It is also known to stereotatically implant

25 a radioactive seed (such as yttrium[90]) to cause deep brain tumor necrosis, as in craniopharyngioma and it is known to carry out stereotactic biopsy of various intracranial tumors, including hypothalamic astrocytoma. Additionally, thalamotomy and/or subthalamotomy or campotomy are known neurosurgical procedures for treating movement

30 disorders, such as dystonia, through stereotactic surgery. Tasker R., *Surgical Treatment of the Dystonias*, chapter 105, pages 1015-1032, in Gildenberg P.L. et al., *Textbook of Stereotactic and Functional*

Neurosurgery, McGraw-Hill (1998). A variety of techniques exist to permit precise guidance to location of a probe, including computed tomography and magnetic resonance imaging. Notably, hypothalamic ablation results in an irreversible destructive brain lesion.

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Therapies such as deep brain electrode stimulation can create problems due to wire erosion, lead friction, infection of the implantable pulse generator, malfunction of the implantable pulse generator, electrical shock and lead migration. Other complications due to

10   electrode stimulation can include dysarthria, disequilibrium, paresis and gait disorder. See e.g. Koller, W.C. et al., *Surgical Treatment of Parkinson's Disease*, J Neurol Sci 167;1-10:1999, and Schuurman P.R., et al., *A Comparison of Continuous Thalamic Stimulation and Thalamotomy for Suppression of Severe Tremor*, NEJM 342(7);461-

15   468:2000.

Aside from surgical ablation or stimulation, external radiotherapy (gamma knife radiosurgery) has also been used for the treatment of a number of drug resistant maladies of intracranial origin. Drawbacks with

20   this procedure are that a positive therapeutic result may not occur until up to eight months after the radiosurgery, and the long term benefits as well as radiation side effects are currently unknown.

Botulinum Toxin

25   The bacterial genus *Clostridium* includes more than one hundred and twenty seven species, grouped according to morphology and function. The anaerobic, gram positive bacterium *Clostridium botulinum* produces a potent polypeptide neurotoxin, botulinum toxin, which causes the neuroparalytic illness in humans and animals referred to as botulism.

30   The spores of Clostridium botulinum are found in soil and can grow in improperly sterilized and sealed food containers of home based canneries, which are the cause of many of the cases of botulism. The

effects of botulism typically appear 18 to 36 hours after eating food infected with a Clostridium botulinum culture or spores. The botulinum toxin can apparently pass unattenuated through the lining of the gut and attack peripheral motor nerves. Symptoms of botulinum toxin

5   intoxication can progress from difficulty walking, swallowing, and speaking to paralysis of the respiratory muscles and death.

Botulinum toxin type A is the most lethal natural biological agent known to man. About 50 picograms of a commercially available

10   botulinum toxin type A (purified neurotoxin complex)[1] is a LD_{50} in mice (i.e. 1 unit). One unit of BOTOX® contains about 50 picograms (about 56 attomoles) of botulinum toxin type A complex. Interestingly, on a molar basis, botulinum toxin type A is about 1.8 billion times more lethal than diphtheria, about 600 million times more lethal than sodium

15   cyanide, about 30 million times more lethal than cobra toxin and about 12 million times more lethal than cholera. Singh, *Critical Aspects of Bacterial Protein Toxins*, pages 63-84 (chapter 4) of Natural Toxins II, edited by B.R. Singh et al., Plenum Press, New York (1976) (where the stated LD_{50} of botulinum toxin type A of 0.3 ng equals 1 U is corrected

20   for the fact that about 0.05 ng of BOTOX® equals 1 unit). One unit (U) of botulinum toxin is defined as the LD_{50} upon intraperitoneal injection into female Swiss Webster mice weighing 18 to 20 grams each.

Seven generally immunologically distinct botulinum neurotoxins have

25   been characterized, these being respectively botulinum neurotoxin serotypes A, B, C_1, D, E, F and G each of which is distinguished by neutralization with type-specific antibodies. The different serotypes of botulinum toxin vary in the animal species that they affect and in the severity and duration of the paralysis they evoke. For example, it has

30   been determined that botulinum toxin type A is 500 times more potent, as measured by the rate of paralysis produced in the rat, than is

botulinum toxin type B. Additionally, botulinum toxin type B has been determined to be non-toxic in primates at a dose of 480 U/kg which is about 12 times the primate LD_{50} for botulinum toxin type A. Moyer E et al., *Botulinum Toxin Type B: Experimental and Clinical Experience*,

5   being chapter 6, pages 71-85 of "Therapy With Botulinum Toxin", edited by Jankovic, J. et al. (1994), Marcel Dekker, Inc. Botulinum toxin apparently binds with high affinity to cholinergic motor neurons, is translocated into the neuron and blocks the release of acetylcholine.

10   Regardless of serotype, the molecular mechanism of toxin intoxication appears to be similar and to involve at least three steps or stages. In the first step of the process, the toxin binds to the presynaptic membrane of a motor neuron through a specific interaction between the heavy (or H) chain of the botulinum toxin and a neuronal cell surface

15   receptor. The receptor is believed to be different for each type of botulinum toxin and for tetanus toxin. The carboxyl end segment of the H chain, H_C, appears to be important for targeting of the toxin to the surface of the motor neuron.

20   In the second step, the toxin crosses the plasma membrane of the motor neuron. The toxin is first engulfed by the cell through receptor-mediated endocytosis, and an endosome containing the toxin is formed. The toxin then escapes the endosome into the cytoplasm of the cell. This step may be mediated by the amino end segment of the H chain,

25   H_N, which triggers a conformational change of the toxin in response to a pH of about 5.5 or lower. Endosomes are known to possess a proton pump which decreases intra-endosomal pH. The conformational shift exposes hydrophobic residues in the toxin, which permits the toxin to embed itself in the endosomal membrane. The toxin (or at a minimum

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[1]   Available from Allergan, Inc., of Irvine, California under the tradename BOTOX® in 100 unit vials)

the light chain) then translocates through the endosomal membrane into the cytoplasm.

The last step of the mechanism of botulinum toxin activity appears to
5   involve reduction of the disulfide bond joining the heavy chain, H chain, and the light (or L) chain of the toxin. The entire toxic activity of botulinum toxin and of the tetanus toxin is contained in the L chain of the holotoxin. The L chain is a zinc (Zn^{++}) endopeptidase which selectively cleaves proteins essential for recognition and docking of
10   neurotransmitter-containing vesicles with the cytoplasmic surface of the plasma membrane, and fusion of the vesicles with the plasma membrane. Tetanus neurotoxin, botulinum toxin types B, D, F, and G cause degradation of synaptobrevin (also called vesicle-associated membrane protein (VAMP)), a synaptosomal membrane protein. Most
15   of the VAMP present at the cytoplasmic surface of the synaptic vesicle is removed as a result of any one of these cleavage events. Botulinum toxin serotype A and E cleave SNAP-25. Botulinum toxin serotype C cleaves syntaxin and SNAP-25. Each of the botulinum toxins specifically cleaves a different bond, except botulinum toxin type B and
20   tetanus toxin which cleave the same bond.

Botulinum toxins have been used in clinical settings for the treatment of neuromuscular disorders characterized by hyperactive skeletal muscles. A botulinum toxin type A complex has been approved by the
25   U.S. Food and Drug Administration for the treatment of blepharospasm, strabismus and hemifacial spasm. Non-type A botulinum toxin serotypes apparently has a lower potency and/or a shorter duration of activity as compared to botulinum toxin type A. Most if not all of the botulinum toxins can, upon intramuscular injection, produce significant
30   muscle paralysis within one day of the injection, as measured, for example, by the mouse Digit Abduction Score (DAS). Aoki K. R., *Preclinical Update on BOTOX (Botulinum Toxin Type A)-Purified*

Neurotoxin Complex Relative to Other Botulinum Toxin Preparations,
Eur J. Neur 1999, 6 (suppl 4):S3-S10. Maximal clinical effect may not
result for several days. The typical duration of symptomatic relief from a
single intramuscular injection of botulinum toxin type A averages about

5   three months. Apparently, a substrate for a botulinum toxin can be
found in a variety of different cell types. See e.g. *Biochem ,J 1;339 (pt
1):159-65:1999*, and *Mov Disord, 10(3):376:1995* (pancreatic islet B
cells contains at least SNAP-25 and synaptobrevin).

10   The molecular weight of the botulinum toxin protein molecule, for all
seven of the known botulinum toxin serotypes, is about 150 kD.
Interestingly, the botulinum toxins are released by Clostridial bacterium
as complexes comprising the 150 kD botulinum toxin protein molecule
along with associated non-toxin proteins. Thus, the botulinum toxin type

15   A complex can be produced by Clostridial bacterium as 900 kD, 500 kD
and 300 kD forms. Botulinum toxin types B and C_1 is apparently
produced as only a 700 kD or 500 kD complex. Botulinum toxin type D
is produced as both 300 kD and 500 kD complexes. Finally, botulinum
toxin types E and F are produced as only approximately 300 kD

20   complexes. The complexes (i.e. molecular weight greater than about
150 kD) are believed to contain a non-toxin hemaglutinin protein and a
non-toxin and non-toxic nonhemaglutinin protein. These two non-toxin
proteins (which along with the botulinum toxin molecule comprise the
relevant neurotoxin complex) may act to provide stability against

25   denaturation to the botulinum toxin molecule and protection against
digestive acids when toxin is ingested. Additionally, it is possible that
the larger (greater than about 150 kD molecular weight) botulinum toxin
complexes may result in a slower rate of diffusion of the botulinum toxin
away from a site of intramuscular injection of a botulinum toxin complex.

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    In vitro studies have indicated that botulinum toxin inhibits potassium
cation induced release of both acetylcholine and norepinephrine from

primary cell cultures of brainstem tissue. Additionally, it has been reported that botulinum toxin inhibits the evoked release of both glycine and glutamate in primary cultures of spinal cord neurons and that in brain synaptosome preparations botulinum toxin inhibits the release of

5 each of the neurotransmitters acetylcholine, dopamine, norepinephrine (Habermann E., et al., *Tetanus Toxin and Botulinum A and C Neurotoxins Inhibit Noradrenaline Release From Cultured Mouse Brain*, J Neurochem 51(2);522-527:1988) CGRP, substance P and glutamate (Sanchez-Prieto, J., et al., *Botulinum Toxin A Blocks Glutamate*

10 *Exocytosis From Guinea Pig Cerebral Cortical Synaptosomes*, Eur J. Biochem 165;675-681:1897. Thus, when adequate concentrations are used, stimulus-evoked release of most neurotransmitters is blocked by botulinum toxin. See e.g. Pearce, L.B., *Pharmacologic Characterization of Botulinum Toxin For Basic Science and Medicine*, Toxicon

15 35(9);1373-1412 at 1393; Bigalke H., et al., *Botulinum A Neurotoxin Inhibits Non-Cholinergic Synaptic Transmission in Mouse Spinal Cord Neurons in Culture*, Brain Research 360;318-324:1985; Habermann E., *Inhibition by Tetanus and Botulinum A Toxin of the release of [³H]Noradrenaline and [³H]GABA From Rat Brain Homogenate*,

20 Experientia 44;224-226:1988, Bigalke H., et al., *Tetanus Toxin and Botulinum A Toxin Inhibit Release and Uptake of Various Transmitters, as Studied with Particulate Preparations From Rat Brain and Spinal Cord*, Naunyn-Schmiedeberg's Arch Pharmacol 316;244-251:1981, and; Jankovic J. et al., *Therapy With Botulinum Toxin*, Marcel Dekker, Inc.,

25 (1994), page 5.

Botulinum toxin type A can be obtained by establishing and growing cultures of Clostridium botulinum in a fermenter and then harvesting and purifying the fermented mixture in accordance with known procedures.

30 All the botulinum toxin serotypes are initially synthesized as inactive single chain proteins which must be cleaved or nicked by proteases to become neuroactive. The bacterial strains that make botulinum toxin

serotypes A and G possess endogenous proteases and serotypes A and G can therefore be recovered from bacterial cultures in predominantly their active form. In contrast, botulinum toxin serotypes C_1, D and E are synthesized by nonproteolytic strains and are therefore

5   typically unactivated when recovered from culture. Serotypes B and F are produced by both proteolytic and nonproteolytic strains and therefore can be recovered in either the active or inactive form. However, even the proteolytic strains that produce, for example, the botulinum toxin type B serotype only cleave a portion of the toxin

10   produced. The exact proportion of nicked to unnicked molecules depends on the length of incubation and the temperature of the culture. Therefore, a certain percentage of any preparation of, for example, the botulinum toxin type B toxin is likely to be inactive, possibly accounting for the known significantly lower potency of botulinum toxin type B as

15   compared to botulinum toxin type A. The presence of inactive botulinum toxin molecules in a clinical preparation will contribute to the overall protein load of the preparation, which has been linked to increased antigenicity, without contributing to its clinical efficacy. Additionally, it is known that botulinum toxin type B has, upon intramuscular injection, a

20   shorter duration of activity and is also less potent than botulinum toxin type A at the same dose level.

High quality crystalline botulinum toxin type A can be produced from the Hall A strain of Clostridium botulinum with characteristics of $\geq 3 \times 10^7$

25   U/mg, an A_{260}/A_{278} of less than 0.60 and a distinct pattern of banding on gel electrophoresis. The known Shantz process can be used to obtain crystalline botulinum toxin type A, as set forth in Shantz, E.J., et al, *Properties and Use of Botulinum Toxin and Other Microbial Neurotoxins in Medicine*, Microbiol Rev. 56;80-99:1992. Generally, the botulinum

30   toxin type A complex can be isolated and purified from an anaerobic fermentation by cultivating *Clostridium botulinum* type A in a suitable medium. The known process can also be used, upon separation out of

the non-toxin proteins, to obtain pure botulinum toxins, such as for example: purified botulinum toxin type A with an approximately 150 kD molecular weight with a specific potency of 1-2 X 10^8 LD_{50} U/mg or greater; purified botulinum toxin type B with an approximately 156 kD

5   molecular weight with a specific potency of 1-2 X 10^8 LD_{50} U/mg or greater, and; purified botulinum toxin type F with an approximately 155 kD molecular weight with a specific potency of 1-2 X 10^7 LD_{50} U/mg or greater.

10   Botulinum toxins and/or botulinum toxin complexes can be obtained from List Biological Laboratories, Inc., Campbell, California; the Centre for Applied Microbiology and Research, Porton Down , U.K.; Wako (Osaka, Japan), Metabiologics (Madison, Wisconsin) as well as from Sigma Chemicals of St Louis, Missouri.

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Both pure botulinum toxin and botulinum toxin complexes can be used to prepare a pharmaceutical composition. Both pure botulinum toxin and botulinum toxin complexes, such a the toxin type A complex are susceptible to denaturation due to surface denaturation, heat, and

20   alkaline conditions. Inactivated toxin forms toxoid proteins which may be immunogenic. The resulting antibodies can render a patient refractory to toxin injection.

As with enzymes generally, the biological activities of the botulinum

25   toxins (which are intracellular peptidases) is dependent, at least in part, upon their three dimensional conformation. Thus, botulinum toxin type A is detoxified by heat, various chemicals surface stretching and surface drying. Additionally, it is known that dilution of the toxin complex obtained by the known culturing, fermentation and purification to the

30   much, much lower toxin concentrations used for pharmaceutical composition formulation results in rapid detoxification of the toxin unless a suitable stabilizing agent is present. Dilution of the toxin from

milligram quantities to a solution containing nanograms per milliliter presents significant difficulties because of the rapid loss of specific toxicity upon such great dilution. Since the toxin may be used months or years after the toxin containing pharmaceutical composition is

5   formulated, the toxin can stabilized with a stabilizing agent such as albumin and gelatin.

A commercially available botulinum toxin containing pharmaceutical composition is sold under the trademark BOTOX® (available from

10   Allergan, Inc., of Irvine, California). BOTOX® consists of a purified botulinum toxin type A complex, albumin and sodium chloride packaged in sterile, vacuum-dried form. The botulinum toxin type A is made from a culture of the Hall strain of Clostridium botulinum grown in a medium containing N-Z amine and yeast extract. The botulinum toxin type A

15   complex is purified from the culture solution by a series of acid precipitations to a crystalline complex consisting of the active high molecular weight toxin protein and an associated hemagglutinin protein. The crystalline complex is re-dissolved in a solution containing saline and albumin and sterile filtered (0.2 microns) prior to vacuum-drying.

20   The vacuum-dried product is stored in a freezer at or below -5°C. BOTOX® can be reconstituted with sterile, non-preserved saline prior to intramuscular injection. Each vial of BOTOX® contains about 100 units (U) of Clostridium botulinum toxin type A purified neurotoxin complex, 0.5 milligrams of human serum albumin and 0.9 milligrams of sodium

25   chloride in a sterile, vacuum-dried form without a preservative.

To reconstitute vacuum-dried BOTOX®, sterile normal saline without a preservative; (0.9% Sodium Chloride Injection) is used by drawing up the proper amount of diluent in the appropriate size syringe. Since

30   BOTOX® may be denatured by bubbling or similar violent agitation, the diluent is gently injected into the vial. For sterility reasons BOTOX® is

preferably administered within four hours after the vial is removed from the freezer and reconstituted. During these four hours, reconstituted BOTOX® can be stored in a refrigerator at about 2° C. to about 8°C. Reconstituted, refrigerated BOTOX® has been reported to retain its

5   potency for at least about two weeks. *Neurology*, 48:249-53:1997.

It has been reported that botulinum toxin type A has been used in clinical settings as follows:

(1) about 75-125 units of BOTOX® per intramuscular injection

10   (multiple muscles) to treat cervical dystonia;

(2) 5-10 units of BOTOX® per intramuscular injection to treat glabellar lines (brow furrows) (5 units injected intramuscularly into the procerus muscle and 10 units injected intramuscularly into each corrugator supercilii muscle);

15   (3) about 30-80 units of BOTOX® to treat constipation by intrasphincter injection of the puborectalis muscle;

(4) about 1-5 units per muscle of intramuscularly injected BOTOX® to treat blepharospasm by injecting the lateral pre-tarsal orbicularis oculi muscle of the upper lid and the lateral pre-tarsal orbicularis oculi of the

20   lower lid.

(5) to treat strabismus, extraocular muscles have been injected intramuscularly with between about 1-5 units of BOTOX®, the amount injected varying based upon both the size of the muscle to be injected and the extent of muscle paralysis desired (i.e. amount of diopter

25   correction desired).

(6) to treat upper limb spasticity following stroke by intramuscular injections of BOTOX® into five different upper limb flexor muscles, as follows:

(a) flexor digitorum profundus: 7.5 U to 30 U

30   (b) flexor digitorum sublimus: 7.5 U to 30 U

(c) flexor carpi ulnaris: 10 U to 40 U

(d) flexor carpi radialis: 15 U to 60 U

(e) biceps brachii: 50 U to 200 U. Each of the five indicated muscles has been injected at the same treatment session, so that the patient receives from 90 U to 360 U of upper limb flexor muscle

5   BOTOX® by intramuscular injection at each treatment session.

(7) to treat migraine, pericranial injected (injected symmetrically into glabellar, frontalis and temporalis muscles) injection of 25 U of BOTOX® has showed significant benefit as a prophylactic treatment of migraine compared to vehicle as measured by decreased measures of migraine

10   frequency, maximal severity, associated vomiting and acute medication use over the three month period following the 25 U injection.

Additionally, intramuscular botulinum toxin has been used in the treatment of tremor in patients with Parkinson's disease, although it has

15   been reported that results have not been impressive. Marjama-Jyons, J., et al., *Tremor-Predominant Parkinson's Disease*, Drugs & Aging 16(4);273-278:2000.

It is known that botulinum toxin type A can have an efficacy for up to

20   12 months (*European J. Neurology* 6 (Supp 4): S111-S1150:1999), and in some circumstances for as long as 27 months. *The Laryngoscope* 109:1344-1346:1999. However, the usual duration of an intramuscular injection of Botox® is typically about 3 to 4 months.

25   The success of botulinum toxin type A to treat a variety of clinical conditions has led to interest in other botulinum toxin serotypes. A study of two commercially available botulinum type A preparations (BOTOX® and Dysport®) and preparations of botulinum toxins type B and F (both obtained from Wako Chemicals, Japan) has been carried out to

30   determine local muscle weakening efficacy, safety and antigenic potential. Botulinum toxin preparations were injected into the head of

the right gastrocnemius muscle (0.5 to 200.0 units/kg) and muscle weakness was assessed using the mouse digit abduction scoring assay (DAS). ED_{50} values were calculated from dose response curves. Additional mice were given intramuscular injections to determine LD_{50}

5 doses. The therapeutic index was calculated as LD_{50}/ED_{50}. Separate groups of mice received hind limb injections of BOTOX® (5.0 to 10.0 units/kg) or botulinum toxin type B (50.0 to 400.0 units/kg), and were tested for muscle weakness and increased water consumption, the later being a putative model for dry mouth. Antigenic potential was assessed

10 by monthly intramuscular injections in rabbits (1.5 or 6.5 ng/kg for botulinum toxin type B or 0.15 ng/kg for BOTOX®). Peak muscle weakness and duration were dose related for all serotypes. DAS ED_{50} values (units/kg) were as follows: BOTOX®: 6.7, Dysport®: 24.7, botulinum toxin type B: 27.0 to 244.0, botulinum toxin type F: 4.3.

15 BOTOX® had a longer duration of action than botulinum toxin type B or botulinum toxin type F. Therapeutic index values were as follows: BOTOX®: 10.5, Dysport®: 6.3, botulinum toxin type B: 3.2. Water consumption was greater in mice injected with botulinum toxin type B than with BOTOX®, although botulinum toxin type B was less effective at

20 weakening muscles. After four months of injections 2 of 4 (where treated with 1.5 ng/kg) and 4 of 4 (where treated with 6.5 ng/kg) rabbits developed antibodies against botulinum toxin type B. In a separate study, 0 of 9 BOTOX® treated rabbits demonstrated antibodies against botulinum toxin type A. DAS results indicate relative peak potencies of

25 botulinum toxin type A being equal to botulinum toxin type F, and botulinum toxin type F being greater than botulinum toxin type B. With regard to duration of effect, botulinum toxin type A was greater than botulinum toxin type B, and botulinum toxin type B duration of effect was greater than botulinum toxin type F. As shown by the therapeutic index

30 values, the two commercial preparations of botulinum toxin type A (BOTOX® and Dysport®) are different. The increased water

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consumption behavior observed following hind limb injection of botulinum toxin type B indicates that clinically significant amounts of this serotype entered the murine systemic circulation. The results also indicate that in order to achieve efficacy comparable to botulinum toxin

5   type A, it is necessary to increase doses of the other serotypes examined. Increased dosage can comprise safety. Furthermore, in rabbits, type B was more antigenic than was BOTOX®, possibly because of the higher protein load injected to achieve an effective dose of botulinum toxin type B. *Eur J Neurol 1999 Nov;6(Suppl 4):S3-S10.*

10

In addition to having pharmacologic actions at the peripheral location, botulinum toxins may also have inhibitory effects in the central nervous system. Work by Weigand et al, *Nauny-Schmiedeberg's Arch. Pharmacol.* 1976; 292, 161-165, and Habermann, *Nauny-*

15   *Schmiedeberg's Arch. Pharmacol.* 1974; 281, 47-56 showed that botulinum toxin is able to ascend to the spinal area by retrograde transport. As such, a botulinum toxin injected at a peripheral location, for example intramuscularly, may be retrograde transported to the spinal cord.

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U.S. Patent No. 5,989,545 discloses that a modified clostridial neurotoxin or fragment thereof, preferably a botulinum toxin, chemically conjugated or recombinantly fused to a particular targeting moiety can be used to treat pain by administration of the agent to the spinal cord.

25

Acetylcholine

Typically only a single type of small molecule neurotransmitter is released by each type of neuron in the mammalian nervous system. The neurotransmitter acetylcholine is secreted by neurons in many

30   areas of the brain, but specifically by the large pyramidal cells of the motor cortex, by several different neurons in the basal ganglia, by the

motor neurons that innervate the skeletal muscles, by the preganglionic neurons of the autonomic nervous system (both sympathetic and parasympathetic), by the postganglionic neurons of the parasympathetic nervous system, and by some of the postganglionic neurons of the

5   sympathetic nervous system. Essentially, only the postganglionic sympathetic nerve fibers to the sweat glands, the piloerector muscles and a few blood vessels are cholinergic as most of the postganglionic neurons of the sympathetic nervous system secret the neurotransmitter norepinephine. In most instances acetylcholine has an excitatory effect.

10   However, acetylcholine is known to have inhibitory effects at some of the peripheral parasympathetic nerve endings, such as inhibition of heart rate by the vagal nerve.

The efferent signals of the autonomic nervous system are

15   transmitted to the body through either the sympathetic nervous system or the parasympathetic nervous system. The preganglionic neurons of the sympathetic nervous system extend from preganglionic sympathetic neuron cell bodies located in the intermediolateral horn of the spinal cord. The preganglionic sympathetic nerve fibers, extending from the

20   cell body, synapse with postganglionic neurons located in either a paravertebral sympathetic ganglion or in a prevertebral ganglion. Since, the preganglionic neurons of both the sympathetic and parasympathetic nervous system are cholinergic, application of acetylcholine to the ganglia will excite both sympathetic and parasympathetic postganglionic

25   neurons.

Acetylcholine activates two types of receptors, muscarinic and nicotinic receptors. The muscarinic receptors are found in all effector cells stimulated by the postganglionic, neurons of the parasympathetic

30   nervous system as well as in those stimulated by the postganglionic cholinergic neurons of the sympathetic nervous system. The nicotinic receptors are found in the adrenal medulla, as well as within the

autonomic ganglia, that is on the cell surface of the postganglionic

neuron at the synapse between the preganglionic and postganglionic

neurons of both the sympathetic and parasympathetic systems.

Nicotinic receptors are also found in many nonautonomic nerve endings,

5   for example in the membranes of skeletal muscle fibers at the

neuromuscular junction.

Acetylcholine is released from cholinergic neurons when small, clear,

intracellular vesicles fuse with the presynaptic neuronal cell membrane.

10   A wide variety of non-neuronal secretory cells, such as, adrenal medulla

(as well as the PC12 cell line) and pancreatic islet cells release

catecholamines and parathyroid hormone, respectively, from large

dense-core vesicles. The PC12 cell line is a clone of rat

pheochromocytoma cells extensively used as a tissue culture model for

15   studies of sympathoadrenal development. Botulinum toxin inhibits the

release of both types of compounds from both types of cells *in vitro*,

permeabilized (as by electroporation) or by direct injection of the toxin

into the denervated cell. Botulinum toxin is also known to block release

of the neurotransmitter glutamate from cortical synaptosomes cell

20   cultures.

A neuromuscular junction is formed in skeletal muscle by the

proximity of axons to muscle cells. A signal transmitted through the

nervous system results in an action potential at the terminal axon, with

25   activation of ion channels and resulting release of the neurotransmitter

acetylcholine from intraneuronal synaptic vesicles, for example at the

motor endplate of the neuromuscular junction. The acetylcholine

crosses the extracellular space to bind with acetylcholine receptor

proteins on the surface of the muscle end plate. Once sufficient binding

30   has occurred, an action potential of the muscle cell causes specific

membrane ion channel changes, resulting in muscle cell contraction.

The acetylcholine is then released from the muscle cells and

metabolized by cholinesterases in the extracellular space. The metabolites are recycled back into the terminal axon for reprocessing into further acetylcholine.

5   Cholinergic Brain Systems

Cholinergic influence of both the motor and visual thalamus originates from both the brainstem and the basal forebrain. See e.g. Billet S., et al., *Cholinergic Projections to the Visual Thalamus and Superior Colliculus*, Brain Res. 847;121-123:1999 and Oakman, S.A. et

10   al., *Characterization of the Extent of Pontomesencephalic Cholinergic Neurons' projections to the Thalamus: Comparison with Projections to Midbrain Dopaminergic Groups*, Neurosci 94(2);529-547;1999. Thus, it is known based on histochemical studies using acetylcholinesterase (AchE) staining and retrograde tracing with choline acetyltransferase

15   (ChAT) immunochemistry that there can be ascending cholinergic stimulation by the brainstem of thalamic neurons. Steriade M. et al., *Brain Cholinergic Systems*, Oxford University Press (1990), chapter 1. Indeed, many thalamic nuclei receive dense cholinergic innervation from brainstem reticular formations. *Ibid*, page 167. Known brainstem

20   cholinergic cell groups are located within: (1) the rostral pons at what is termed a Ch5 location, which is located within the central tegmental field around the brachium conjunctivum, forming a pedunculopontine tegmental nucleus, and; (2) the caudal part of the midbrain, at what is termed a Ch6 location, the laterodorsal tegmental nucleus, which is

25   embedded in the periaqueductal and periventricular gray matter. The Ch5 and Ch6 cell groups can consist almost exclusively of cholinergic neurons and together form the pontine cholinergic system. The Ch5-Ch6 cholinergic groups provide direct ascending projections that terminate in a number of target structure in the midbrain, diencephalon

30   and telencephalon, including the superior colliculus, anterior pretectal area, interstitial magnocellular nucleus of the posterior commissure, lateral habenular nucleus, thalamus, magnocellular preoptic nucleus,

lateral mammillary nucleus, basal forebrain, olfactory bulb, medial prefrontal cortex and pontine nuclei. Stone T.W., *CNS Neurotransmitters and Neuromodulators: Acetylcholine*, CRC Press (19950, page 16. See also Schafer M. K.-H. et al., *Cholinergic Neurons*

5   *and Terminal Fields Revealed by Immunochemistry for the Vesicular Acetylcholine Transporter. I. Central Nervous System*, Neuroscience, 84(2);331-359:1998. Three dimensional localization of Ch1-8 cholinergic nuclei have been mapped in humans. See e.g. Tracey, D.J., et al., *Neurotransmitters in the Human Brain*, Plenum Press (1995),

10   pages 136-139.

     Additionally, the basal forebrain (proencephalon) provides cholinergic innervation of the dorsal thalamus, as well as to the neocortex, hippocampus, amygdala and olfactory bulb. See e.g. Steridae, page

15   136-136. Basal forebrain areas where the great proportion of neurons are cholinergic include the medial septal nucleus (Ch1), the vertical branches of the diagonal band nuclei (Ch2), the horizontal branches of the diagonal band nuclei (Ch3), and the magnocellular nucleus basalis (Ch4), which is located dorsolaterally to the Ch3 cell group. Ch1 and

20   Ch2 provide the major component of cholinergic projection to the hippocampus. The cells in the Ch3 sector project to the olfactory bulb.

     Furthermore, cholinergic neurons are present in the thalamus. Rico, B. et al., *A Population of Cholinergic Neurons is Present in the Macaque*

25   *Monkey Thalamus*, Eur J Neurosci, 10;2346-2352:1998.

     Abnormalities in the brain's cholinergic system have been consistently identified in a variety of neuropsychiatric disorders including Alzheimer's disease, Parkinson's disease and dementia with Lewy

30   bodies. Thus, in Alzheimer's disease there is hypoactivity of cholinergic projections to the hippocampus and cortex. In individuals with dementia with Lewy bodies extensive neocortical cholinergic deficits are

believed to exist and in Parkinson's disease there is a loss of pedunculopontine cholinergic neurons. Notably, *in vivo* imaging of cholinergic activity in the human brain has been reported. Perry, et al., *Acetylcholine in Mind: a Neurotransmitter Correlate of Consciousness?*,

5   TINS 22(6);273-280:1999.

What is needed therefore is a method for effectively treating an endocrine disorder by administration of a pharmaceutical which has the characteristics of long duration of activity, low rates of diffusion out of a

10   chosen intracranial target tissue where administered, and nominal systemic effects at therapeutic dose levels.

SUMMARY

15   The present invention meets this need and provides methods for effectively treating a variety of endocrine disorders by intracranial administration of a neurotoxin which has the characteristics of long duration of activity, low rates of diffusion out of an intracranial site where administered and insignificant systemic effects at therapeutic dose

20   levels.

The following definitions apply herein:

"About" means approximately or nearly and in the context of a

25   numerical value or range set forth herein means ±10% of the numerical value or range recited or claimed.

"Biological activity" includes, with regard to a neurotoxin, the ability to influence synthesis, exocytosis, receptor binding and/or uptake of a

30   neurotransmitter, such as acetylcholine, or of an neuroendocrine secretory product, such as a hypothalamic regulatory factor.

"Botulinum toxin" includes pure botulinum toxin (i.e. the approximately 150 kD toxin molecule) and botulinum toxin complexes (i.e. the pure botulinum toxin molecule in association with non-toxin molecules, such as haemagglutins, to form a complex of up to about

5   900 kD).

"Local administration" means direct administration of a pharmaceutical at or to the vicinity of a site on or within an animal body, at which site a biological effect of the pharmaceutical is desired. Local

10   administration excludes systemic routes of administration, such as intravenous or oral administration.

"Neurotoxin" means a biologically active molecule with a specific affinity for a neuronal cell surface receptor. Neurotoxin includes

15   Clostridial toxins both as pure toxin and as complexed with one to more non-toxin, toxin associated proteins

"Intracranial" means within the cranium or at or near the dorsal end of the spinal cord and includes the medulla, brain stem, pons, cerebellum

20   and cerebrum. The hypophysis and the hypothalamus are both considered to be intracranial.

A method for treating an endocrine disorder according to the present invention can comprise the step of intracranial administration of a

25   neurotoxin to a patient, thereby alleviating a symptom of an endocrine disorder. The neurotoxin can be made, for example, by a bacterium selected from the group consisting of *Clostridium botulinum, Clostridium butyricum, Clostridium beratti*, and *Clostridium tetani* or can be expressed by a suitable host (i.e. a recombinantly altered E. coli) which

30   encodes for a neurotoxin made non-recombinantly by a *Clostridium botulinum, Clostridium butyricum, Clostridium beratti or Clostridium tetani.* Preferably, the neurotoxin is a botulinum toxin, such as a

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botulinum toxin type A, B, C_1, D, E, F and G. More preferably, the botulinum toxin is botulinum toxin type A because of the high potency, ready availability and long history of clinical use of botulinum toxin type A to treat various disorders.

5

The botulinum toxin can be locally administered in an amount of between about 10^{-2} units and about 500 units and the endocrine disorder symptom alleviating effect from a local administration of a botulinum toxin according to the present invention can persist for

10 between about 1 month and about 5 years.

Notably, the neurotoxin can be administered to the hypothalamus, such as to the median eminence region and/or arcuate nucleus area of the hypothalamus. Additionally, the neurotoxin can be administered to

15 the pituitary gland, including to the anterior pituitary or posterior pituitary. The intracranial administration step can comprise the step of implantation of a controlled release botulinum toxin implant.

A detailed embodiment of a method within the scope of the present

20 invention for treating an endocrine disorder can comprise the step of intracranial administration of a therapeutically effective amount of a botulinum toxin to a patient, thereby treating a symptom of an endocrine disorder. The endocrine disorders treatable by a method according to the present invention include afflictions which result from an excess of

25 any of the hypothalamic releasing factors or of any of the pituitary hormones, including GnRH, GH, LH and/or FSH. Thus endocrine disorders such as, for example, acromegaly, gigantism, Cushings disease, hypergonadism and hyperthyroidism, can be treated by a method according to the present invention.

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A further detailed embodiment of a method within the scope of the present invention for treating an endocrine disorder can comprise the

steps of: selecting a neurotoxin (such as a botulinum toxin)with
hypothalamic releasing hormone, or pituitary hormone, suppressant
activity: choosing a hypothalamic or pituitary target tissue which
influences an endocrine disorder; and; intracranially administering to the

5   target tissue a therapeutically effective amount of the neurotoxin
selected, thereby treating the endocrine disorder.

A method for treating hypergonadism according to the present
invention can comprise the step of *in vivo* local administration of a

10   therapeutically effective amount of a botulinum toxin type A to a
cholinergically influenced hypothalamic tissue to a human patient,
thereby alleviating a symptom of hypergonadism in the patient.

Additionally, a contraceptive method according to the present

15   invention can comprise the step of intracranial administration of a
botulinum toxin (such as a botulinum toxin type A) to a patient, thereby
reducing an intracranial secretion of a hormone required for
gametogenesis. Thus, ovulation can be inhibited by intracranial
administration of a botulinum toxin to a patient, thereby reducing an

20   intracranial secretion of a hormone which influences ovulation.
Furthermore, sperm production can be inhibited by intracranial
administration of a botulinum toxin to a patient, thereby reducing an
intracranial secretion of a hormone which influences production.

25   The neurotoxin can be a modified neurotoxin, that is a neurotoxin
which has at least one of its amino acids deleted, modified or replaced,
as compared to a native or the modified neurotoxin can be a
recombinant produced neurotoxin or a derivative or

30   I have surprising found that a botulinum toxin, such as botulinum
toxin type A, can be intracranially administered in amounts between
about 10^{-2} units to about 500 units to alleviate an endocrine disorder

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experienced by a human patient. Preferably, the botulinum toxin used is intracranially administered in an amount of between about 10^{-1} unit and about 50 units. More preferably, the botulinum toxin is administered in an amount of between about 10^{-1} units and about 5 units. Most

5 preferably, from about 0.01 units to about 10 units of a botulinum toxin type A and from about 0.1 to about 100 units of a botulinum toxin type B is used is a method practiced according to the present disclosed invention. With regard to the other botulinum toxin serotypes (including toxin types E and F) the unit dosage to be used is within the range of

10 about 0.01 units to about 500 units, as set forth herein. Significantly, the endocrine disorder alleviating effect of the present disclosed methods can persist for between about 2 months to about 6 months when administration is of aqueous solution of the neurotoxin, and for up to about five years when the neurotoxin is administered as a controlled

15 release implant.

Another preferred method within the scope of the present invention is a method for improving patient function, the method comprising the step of intracranially administering a neurotoxin to a patient, thereby

20 improving patient function as determined by improvement in one or more of the factors of reduced pain, reduced time spent in bed, increased ambulation, healthier attitude and a more varied lifestyle.

25 <u>DESCRIPTION</u>
The present invention is based on the discovery that one or more endocrine disorders can be effectively treated by intracranial, *in vivo*, local administration of an neurotoxin to a human patient. Generally, hormonal excess results in a pathological condition and one aspect of

30 the present invention is directed to methods for treating hormone excess resulting from overproduction of a hypothalamic releasing factor or of a pituitary hormone. The present invention is also based on the discovery

that gametogenesis, and hence conception, can be inhibited by intracranial, *in vivo*, local administration of an neurotoxin to a male or female human patient.

5   Thus, the present invention reveals that significant and lost lasting relief from a variety of different endocrine disorders can be achieved by intracranial administration of a neurotoxin. Intracranial administration permits the blood brain barrier to be bypassed and delivers much more toxin to the brain than is possible by a systemic route of administration.

10   Furthermore, systemic administration of a neurotoxin, such as a botulinum toxin, is contraindicated due to the severe complications (i.e. botulism) which can result from entry of a botulinum toxin into the general circulation. Additionally, since botulinum toxin does not penetrate the blood brain barrier to any significant extent, systemic

15   administration of a botulinum toxin has no practical application to treat an intracranial target tissue.

       According to the present, *in vivo* local administration of a neurotoxin, such as a botulinum toxin, can be used to treat a variety of conditions

20   which can benefit from inhibition of the hypothalamic-pituitary-gonadal axis such as hypergonadotrophic hypergonadism. Thus, a method according to the present invention can be used to treat hypergonadotrophic hypergonadism by decreasing a hypothalamic or pituitary secretion.

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       Additionally, a method according to the present can be used to influence the secretion of gonadotropin releasing hormone (GnRH) by locally administering a preparation influencing central cholinergic activity. In brief, the present invention provides methods for treatment of

30   conditions that can benefit from inhibition of the hypothalamic-pituitary-gonadal axis, for prevention gametogenesis (i.e. inhibition of ovulation or

of sperm production – hence contraception) and to treat hypergonadotrophic hypergonadism.

Without wishing to be bound by theory it can be hypothesized that a mechanism by which a method according to the present invention achieves a desired result is due to inhibition of a cholinergic influence over an endocrine hypothalamic or pituitary tissue by the disclosed local administration of a neurotoxin. Inhibition of a hypothalamic cholinergic influence results, for example, in a decreased release of hypothalamic GnRH, which in turn causes a reduced anterior pituitary release of LH and FSH. A reduction is circulating LH and FSH release can be an effective means of inhibiting conception, and treating various hypergonadic conditions. Thus, a neurotoxin, such as a botulinum toxin, can inhibit neuronal exocytosis of several different CNS neurotransmitters, in particular acetylcholine. It is known that cholinergic neurons are present in both the hypothalamus and in the pituitary. Thus, target tissues for a method within the scope of the present invention can include neurotoxin induced reversible denervation of intracranial areas, such as the hypothalamus. For example, injection or implantation of a neurotoxin to a cholinergically innervated hypothalamic nuclei (such as the arcuate nucleus) can result in a suppression of GHRH and thus GH secretion due to the action of the toxin upon cholinergic terminals projecting into the hypothalamus, and; (2) attenuation of hypothalamic regulatory hormone output due to the action of the toxin upon hypothalamic somata, both cholinergic and non-cholinergic, thereby producing a chemical and therapeutic hypothalamotomy.

As disclosed, the present invention relates to use of a neurotoxin to modulate the hypothalamic-pituitary-gonadal axis through a direct, local administration of a neurotoxin to the hypothalamus and or to the pituitary. In particular, the present invention relates to local, central

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administration of a neurotoxin to modulate the hypothalamic-pituitary-gonadal axis by inhibiting the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus.

5   Suitable neurotoxins for use in the present invention include non-cytotoxic (i.e. no significant neuron or other cell death/necrosis) neurotoxins which can reversibly inhibit one or hypothalamic and/or pituitary endocrine secretions without significant effect, upon other non-hypothalamic or non-pituitary target tissue. Suitable compounds for this

10   purpose are the botulinum toxins.

       The present invention encompasses any suitable method for intracranial administration of a neurotoxin to a selected target tissue, including injection of an aqueous solution of a neurotoxin and

15   implantation of a controlled release system, such as a neurotoxin incorporating polymeric implant at the selected target site. Use of a controlled release implant reduces the need for repeat injections. Intracranial implants are known. For example, brachytherapy for malignant gliomas can include stereotatically implanted, temporary,

20   iodine-125 interstitial catheters. Scharfen. C.O., et al., *High Activity Iodine-125 Interstitial Implant For Gliomas*, Int. J. Radiation Oncology Biol Phys 24(4);583-591:1992. Additionally, permanent, intracranial, low dose ^{125}I seeded catheter implants have been used to treat brain tumors. Gaspar, et al., *Permanent [125]I Implants for Recurrent Malignant*

25   *Gliomas*, Int J Radiation Oncology Biol Phys 43(5);977-982:1999. See also chapter 66, pages 577-580, Bellezza D., et al., *Stereotactic Interstitial Brachytherapy*, in Gildenberg P.L. et al., *Textbook of Stereotactic and Functional Neurosurgery*, McGraw-Hill (1998).

30   Furthermore, local administration of an anti cancer drug to treat malignant gliomas by interstitial chemotherapy using surgically implanted, biodegradable implants is known. For example, intracranial

administration of 3-bis(chloro-ethyl)-1-nitrosourea (BCNU) (Carmustine)

containing polyanhydride wafers, has found therapeutic application.

Brem, H. et al., *The Safety of Interstitial Chemotherapy with BCNU-*

Loaded Polymer Followed by Radiation Therapy in the Treatment of

5   *Newly Diagnosed Malignant Gliomas: Phase I Trial*, J Neuro-Oncology

26:111-123:1995.

A polyanhydride polymer, Gliadel® (Stolle R & D, Inc., Cincinnati, OH)

a copolymer of poly-carboxyphenoxypropane and sebacic acid in a ratio

10   of 20:80 has been used to make implants, intracranially implanted to

treat malignant gliomas. Polymer and BCNU can be co-dissolved in

methylene chloride and spray-dried into microspheres. The

microspheres can then be pressed into discs 1.4 cm in diameter and 1.0

mm thick by compression molding, packaged in aluminum foil pouches

15   under nitrogen atmosphere and sterilized by 2.2 megaRads of gamma

irradiation. The polymer permits release of carmustine over a 2-3 week

period, although it can take more than a year for the polymer to be

largely degraded. Brem, H., et al, *Placebo-Controlled Trial of Safety*

and Efficacy of Intraoperative Controlled Delivery by Biodegradable

20   *Polymers of Chemotherapy for Recurrent Gliomas*, Lancet 345;1008-

1012:1995.

An implant can be prepared by mixing a desired amount of a

stabilized neurotoxin (such as non-reconstituted BOTOX®) into a

25   solution of a suitable polymer dissolved in methylene chloride, at room

temperature. The solution can then transferred to a Petri dish and the

methylene chloride evaporated in a vacuum desiccator. Depending

upon the implant size desired and hence the amount of incorporated

neurotoxin, a suitable amount of the dried neurotoxin incorporating

30   implant is compressed at about 8000 p.s.i. for 5 seconds or at 3000

p.s.i. for 17 seconds in a mold to form implant discs encapsulating the

neurotoxin. See e.g. Fung L. K. et al., *Pharmacokinetics of Interstitial*

Delivery of Carmustine 4-Hydroperoxycyclophosphamide and Paclitaxel From a Biodegradable Polymer Implant in the Monkey Brain, Cancer Research 58;672-684:1998. Diffusion of biological activity of a botulinum toxin within a tissue appears to be a function of dose and can

5   be graduated. Jankovic J., et al *Therapy With Botulinum Toxin*, Marcel Dekker, Inc., (1994), page 150.

Local, intracranial delivery of a neurotoxin, such as a botulinum toxin, can provide a high, local therapeutic level of the toxin and can

10   significantly prevent the occurrence of any systemic toxicity since many neurotoxins, such as the botulinum toxins are too large to cross the blood brain barrier. A controlled release polymer capable of long-term, local delivery of a neurotoxin to an intracranial site can circumvent the restrictions imposed by systemic toxicity and the blood brain barrier, and

15   permit effective dosing of an intracranial target tissue. Suitable neurotoxin and botulinum toxin implants for use in the present invention, are set forth in co-pending U.S. patent applications serial number 09/587250 entitled "Neurotoxin Implant" and serial number 09/624003, entitled "Botulinum Toxin Implant". Such an implant permits direct

20   introduction of a chemotherapeutic agent to a brain target tissue via a controlled release polymer. The implant polymers used are preferably hydrophobic so as to protect the polymer incorporated neurotoxin from water induced decomposition until the toxin is released into the target tissue environment.

25

Local intracranial administration of a botulinum toxin, according to the present invention, by injection or implant to e.g. the cholinergic hypothalamus presents as a superior alternative to hypothalamotomy in the management of *inter alia* hypergonadothophism.

30

A method within the scope of the present invention includes stereotactic placement of a neurotoxin containing implant using the

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Riechert-Mundinger unit and the ZD (Zamorano-Dujovny) multipurpose
localizing unit. A contrast-enhanced computerized tomography (CT)
scan, injecting 120 ml of omnipaque, 350 mg iodine/ml, with 2 mm slice
thickness can allow three dimensional multiplanar treatment planning

5   (STP, Fischer, Freiburg, Germany). This equipment permits planning on
the basis of magnetic resonance imaging studies, merging the CT and
MRI target information for clear target confirmation.

The Leksell stereotactic system (Downs Surgical, Inc., Decatur, GA)

10   modified for use with a GE CT scanner (General Electric Company,
Milwaukee, WI) as well as the Brown-Roberts-Wells (BRW) stereotactic
system (Radionics, Burlington, MA) have been used for this purpose.
Thus, on the morning of the implant, the annular base ring of the BRW
stereotactic frame can be attached to the patient's skull. Serial CT

15   sections can be obtained at 3 mm intervals though the (target tissue)
region with a graphite rod localizer frame clamped to the base plate. A
computerized treatment planning program can be run on a VAX 11/780
computer (Digital Equipment Corporation, Maynard, MA) using CT
coordinates of the graphite rod images to map between CT space and

20   BRW space.

Preferably, a neurotoxin used to practice a method within the scope
of the present invention is a botulinum toxin, such as one of the
serotype A, B, C, D, E, F or G botulinum toxins. Preferably, the

25   botulinum toxin used is botulinum toxin type A, because of its high
potency in humans, ready availability, and known use for the treatment
of skeletal and smooth muscle disorders when locally administered by
intramuscular injection. Botulinum toxin type B is a less preferred
neurotoxin to use in the practice of the disclosed methods because type

30   B is known to have a significantly lower potency and efficacy as
compared, to type A, is not readily available, and has a limited history of
clinical use in humans. Furthermore, the higher protein load with regard

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to type B can cause immunogenic reaction to occur with development of antibodies to the type B neurotoxin.

The amount of a neurotoxin selected for intracranial administration to a target tissue according to the present disclosed invention can be varied based upon criteria such as the endocrine disorder being treated, its severity, the extent of brain tissue involvement or to be treated, solubility characteristics of the neurotoxin toxin chosen as well as the age, sex, weight and health of the patient. For example, the extent of the area of brain tissue influenced is believed to be proportional to the volume of neurotoxin injected, while the quantity of the hypothalamic or pituitary secretion suppressant effect is, for most dose ranges, believed to be proportional to the concentration of neurotoxin injected. Methods for determining the appropriate route of administration and dosage are generally determined on a case by case basis by the attending physician. Such determinations are routine to one of ordinary skill in the art (see for example, *Harrison's Principles of Internal Medicine* (1998), edited by Anthony Fauci et al., 14th edition, published by McGraw Hill).

I have found that a neurotoxin, such as a botulinum toxin, can be intracranially administered according to the present disclosed methods in amounts of between about 10^{-2} units to about 500 units. A dose of about 10^{-2} units can result in a gonadotrophin suppressant effect if delivered a small nuclei. Intracranial administration of less than about 10^{-2} units does not result in a significant or lasting therapeutic result. An intracranial dose of more than 500 units of a neurotoxin, such as a botulinum toxin, poses a significant risk of denervation of sensory or desirable motor functions of neurons adjacent to the target.

Less than about 10^{-2} U/kg can result in a relatively minor, though still observable, gonadotrophin suppressant effect. A more preferred range for intracranial administration of a botulinum toxin, such as botulinum

toxin type A, so as to achieve a gonadotrophin suppressant effect in the patient treated is from about 10^{-1} U/kg to about 50 units. Less than about 10^{-1} U/kg can result in the desired therapeutic effect being of less than the optimal or longest possible duration. A most preferred range

5 for intracranial administration of a botulinum toxin, such as botulinum toxin type A, so as to achieve a desired gonadotrophin suppressant effect in the patient treated is from about 0.1 units to about 10 units. Intracranial administration of a botulinum toxin, such as botulinum toxin type A, in this preferred range can provide dramatic therapeutic

10 success.

The present invention includes within its scope the use of any neurotoxin which has a long duration gonadotrophin suppressant effect when locally applied intracranially to the patient. For example,

15 neurotoxins made by any of the species of the toxin producing Clostridium bacteria, such as *Clostridium botulinum, Clostridium butyricum, and Clostridium beratti* can be used or adapted for use in the methods of the present invention. Additionally, all of the botulinum serotypes A, B, C_1, D, E , F and G can be advantageously used in the

20 practice of the present invention, although type A is the most preferred and type B the least preferred serotype, as explained above. Practice of the present invention can provide a gonadotrophin suppressant effect, per injection, for 3 months or longer in humans.

25 Significantly, a method within the scope of the present invention can provide improved patient function. "Improved patient function" can be defined as an improvement measured by factors such as a reduced pain, reduced time spent in bed, increased ambulation, healthier attitude, more varied lifestyle and/or healing permitted by normal muscle

30 tone. Improved patient function is synonymous with an improved quality of life (QOL). QOL can be assesses using, for example, the known SF-12 or SF-36 health survey scoring procedures. SF-36 assesses a

patient's physical and mental health in the eight domains of physical functioning, role limitations due to physical problems, social functioning, bodily pain, general mental health, role limitations due to emotional problems, vitality, and general health perceptions. Scores obtained can

5   be compared to published values available for various general and patient populations.

As set forth above, I have discovered that a surprisingly effective and long lasting treatment of an endocrine disorder can be achieved by

10   intracranial administration of a neurotoxin to an afflicted patient. In its most preferred embodiment, the present invention is practiced by intracranial injection or implantation of botulinum toxin type A.

The present invention does include within its scope: (a) neurotoxin

15   obtained or processed by bacterial culturing, toxin extraction, concentration, preservation, freeze drying and/or reconstitution and; (b) modified or recombinant neurotoxin, that is neurotoxin that has had one or more amino acids or amino acid sequences deliberately deleted, modified or replaced by known chemical/biochemical amino acid

20   modification procedures or by use of known host cell/recombinant vector recombinant technologies, as well as derivatives or fragments of neurotoxins so made.

Botulinum toxins for use according to the present invention can be

25   stored in lyophilized, vacuum dried form in containers under vacuum pressure or as stable liquids. Prior to lyophilization the botulinum toxin can be combined with pharmaceutically acceptable excipients, stabilizers and/or carriers, such as albumin. The lyophilized material can be reconstituted with saline or water.

30

EXAMPLES

The following examples set forth specific methods encompassed by the present invention to treat an endocrine disorder or to inhibit contraception and are not intended to limit the scope of the invention.

5

A preferred method for local administration of a neurotoxin to a pituitary target tissue according to the present invention is the transphenoidal approach. This surgical approached to the pituitary is carried out by, after preping the patient, including steroid cover, making

10   an incision in the upper gum. The nasal mucosa is stripped from the septum and the pituitary fossa approached through the sphenoid sinus.

For a stereotactic approach (i.e. to the endocrine hypothalamus), a suitable stereotaxis needle (2"-6" long, stiff, hollow bore, non-coring, with

15   an outside diameter of less than one millimeter, for recordation, stimulation and administration of a neurotoxin, such as a botulinum toxin) can be obtained from Popper & Sons (New Hyde Park, New York) or from Surgical911.Com (Old Saybrook, Connecticut). A preferred stereotactic needle is 32guage (32G, 0.009"/0.229mm O.D.) (Popper &

20   Sons). With stereotaxis, endotracheal general anesthesia is followed by making a frontal burr hole at the coronal suture. A fine catheter is used for ventriculography with air or a small amount of contract medium. Using the anterior and posterior commissures as landmarks, a fine needle, 0.8 mm diameter is stereotactically inserted into the target point

25   located at, for example the arcuate nucleus using a CRW apparatus. Use of the Axon Clinical Micropositioner (Axon, Instruments, Inc., Foster City, California) in conjunction with either the Leksell-G or Radionics-CRW sterotaxis frame permits needle placement at or within 50 microns of a desired intracranial target site. Electroencephalography (EEG),

30   electrocorticography (ECG) and electromyography (EMG) of the neck muscles, blood pressure and respiration are monitored. After confirming

the proper placement of the needle into the target point by means of radiograph and stimulating studies, 0.5 units of a botulinum toxin type A (such as Botox) can be injected or an implant capable of releasing about 0.5 units of the botulinum toxin over a 3-4 month period for a continuos

5   period of 2-5 years is implanted at the target tissue site. The desired treatment zone is 2-3 mm in diameter. The number of passes to target is limited to an absolute minimum.

Example 1

10   ## Intracranial Target Tissue Localization and Methodology
Stereotactic procedures can be used for precise intracranial administration of neurotoxin in aqueous form or as an implant to downregulate a hypertrophic endocrine hypothalamus or pituitary. Thus, intracranial administration of a neurotoxin to treat an endocrine can be

15   carried out as follows.

A preliminary MRI scan of the patient can be carried out to obtain the length of the anterior commissure-posterior commissure line and its orientation to external bony landmarks. The base of the frame can then

20   be aligned to the plane of the anterior commissure-posterior commissure line. CT guidance is used and can be supplemented with ventriculography. The posterior commissure can be visualized on 2-mm CT slices and used as a reference point.

25   Physiological corroboration of target tissue localization can be by use of high and low frequency stimulation through a electrode accompanying or incorporated into the long needle syringe used. A thermistor electrode 1.6 mm in diameter with a 2 mm exposed tip can be used (Radionics, Burlington, Massachusetts). With electrode high frequency

30   stimulation (75 Hz) paraesthetic responses can be elicited in the forearm and hand at 0.5-1.0 V using a Radionics lesion generator (Radionics

Radiofrequency Lesion Generator Model RFG3AV). At low frequency (5 Hz) activation or disruption of tremor in the affected limb occurred at 2-3 V. With the methods of the present invention, the electrode is not used to create a lesion. Following confirmation of target tissue localization, a

5   neurotoxin can be injected, thereby causing a reversible, chemical hypothalamectomy. A typical injection is the desired number of units (i.e. about 0.1 to about 5 units of a botulinum toxin type A complex in about 0.01 ml to about 0.1 ml of water or saline. A low injection volume can be uses to minimize toxin diffusion away from target. Typically, the

10 hypothalamic releasing factor or pituitary hormone release inhibition effect can be expected to wear off within about 2-4 months. Thus, an alternate neurotoxin format, neurotoxin incorporated within a polymeric implant, can be used to provide controlled, continuous release of therapeutic amount of the toxin at the desired location over a prolonged

15 period.(i.e. from about 1 year to about 6 years), thereby obviating the need for repeated toxin injections.

Several methods can be used for stereotactically guided injection of a neurotoxin to various intracranial targets, such as the arcuate nucleus

20 (AN) for treatment of acromegaly. Thus a stereotactic magnetic resonance (MRI) method relying on three-dimensional (3D) T1-weighted images for surgical planning and multiplanar T2-weighted images for direct visualization of the AN, coupled with electrophysiological recording and injection guidance for AN injection can be used. See e.g.

25 Bejjani, B.P., et al., *Bilateral Subthalamic Stimulation for Parkinson's Disease by Using Three-Dimensional Stereotactic Magnetic Resonance Imaging and Electrophysiological Guidance,* J Neurosurg 92(4);615-25:2000. The coordinates of the center of the AN can be determined with reference to the patient's anterior commissure-posterior

30 commissure line and a brain atlas.

Electrophysiological monitoring through several parallel tracks can be performed simultaneously to define the functional target accurately. The central track, which is directed at the predetermined target by using MRI imaging, can be selected for neurotoxin injection. No surgical

5   complications are expected.

Computer-aided atlas-based functional neurosurgery methodology can be used to accurately and precisely inject the desired neurotoxin or implant a neurotoxin controlled release implant. Such methodologies

10   permit three-dimensional display and real-time manipulation of hypothalamic structures. Neurosurgical planning with mutually preregistered multiple brain atlases in all three orthogonal orientations is therefore possible and permits increased accuracy of target definition for neurotoxin injection or implantation, reduced time of the surgical

15   procedure by decreasing the number of tracts, and facilitates planning of more sophisticated trajectories. See e.g. Nowinski W.L. et al., *Computer-Aided Stereotactic Functional Neurosurgery Enhanced by the Use of the Multiple Brain Atlas Database*, IEEE Trans Med Imaging 19(1);62-69:2000.

20

Example 2
Treatment of Acromegaly With A Botulinum Toxin
A 55 year old male presents with fatigue, headache, increased

25   sweating, heat intolerance and weight gain. It is determined from a review of photographs supplied by the patient taken over the past 15 years and from the comments of relatives that there has been soft tissue and bone enlargement, with resulting increased hand, foot and hat size, prognathism, enlargement of the tongue, a widening spacing of the teeth

30   and coarsening of facial features. Glucose suppressed GH measured 90 minutes after administration of 100 g glucose is 11 µg/L and a diagnosis of acromegaly is made. Lack of a pituitary adenoma upon

MRI and coned-down x-rays of the sella turcica, and no ectopic GHRH tumors, leads to a conclusion of hypothalamic GHRH excess.

5   Using CAT scan or MRI assisted stereotaxis, as set forth in Example 1 above, 0.5 units of a botulinum toxin type A (such as BOTOX® or about 2 units of Dysport®) is injected into the arcuate nucleus of the hypothalamus. About one week after surgery, GH in response to glucose suppression has fallen to <2μg/L and the excess soft tissue begins to recede and disappear. For extended therapeutic relief, a

10   polymeric implants incorporating a suitable quantity of a botulinum toxin type A can be placed at the target tissue site. The implant can comprise a neurotoxin, such as a of botulinum toxin type A, incorporated within biodegradable polymeric microspheres or a biodegradable pellet, either implant format containing about 10 total units (about 0.5 ng) of the type

15   A toxin (i.e. BOTOX®) with implant characteristics of continuous release over a period of at least about four years of a therapeutic level of the toxin at point of the implant release site and for a radius of about 2-3 mm on each side o the target site. The implant can release about 0.5 unit of toxin essentially immediately and further amounts of about 0.5

20   unit cumulatively over subsequent 2-4 months periods. The same protocol set forth in this example can be used to treat gigantism with the same primary hypothalamic etiology in adolescents.

25   Similarly, as an alternative to use of a botulinum toxin type A, from about 5 to about 50 units of a botulinum toxin type B preparation, or from about 0.1 units to about 100 units of a botulinum toxin type C_1, D, E, F or G can be stereotactically administered and again, for extended therapeutic relief, one or more polymeric implants incorporating a suitable quantity of a botulinum toxin type B, C_1, D, E, F or G can be

30   placed at the target tissue site.

Acromegaly primary to a cholinergically innervated pituitary adenoma can be treated by local administration, or implantation, through transphenoidal surgery of a therapeutically effective amount of a botulinum toxin, as set forth above.

5

Example 3
Treatment of Hyperthyroidism With A Botulinum Toxin

A 29 year old female with high free thyroxin levels and diffuse goiters
10 with high radioactive uptake, but lacking the eye or skin changes characteristic of Graves' disease is diagnosed with hyperthyroidism due to pituitary hyperplasia. Treatment is by transphenoidal surgery to inject 0.5 units of a botulinum toxin type A (such as BOTOX® or about 2 units of Dysport®) into the anterior pituitary. About one week after surgery,
15 circulating thyroxine levels have returned to normal. For extended therapeutic relief, a polymeric implants incorporating a suitable quantity of a botulinum toxin type A can be placed at the target tissue site.

Similarly, as an alternative to use of a botulinum toxin type A, from
20 about 5 to about 50 units of a botulinum toxin type B preparation, or from about 0.1 units to about 100 units of a botulinum toxin type C_1, D, E, F or G can be administered. For extended therapeutic relief, one or more polymeric implants incorporating a suitable quantity of a botulinum toxin type B, C_1, D, E, F or G can be placed at the target tissue site.

25

Example 4
Treatment of Cushing's Disease With A Botulinum Toxin

A 44-year-old male patient is admitted with symptoms of corticol excess
30 including central distribution of adipose tissue, muscle weakness, purplish striae, hypertension, decreased libido and fatigue, determined to be primary to pituitary overproduction of ACTH. Using CAT scan or

MRI assisted stereotaxis, as set forth in Example 1 above, 0.5 units of a botulinum toxin type A (such as BOTOX® or about 2 units of Dysport®) is injected into the hypothalamic nucleus responsible for corticotrophin releasing hormone (CRH) production. Alternately, transphenoidal

5   surgery to locally administer the botulinum toxin to the anterior pituitary can be carried out. Within a few days after surgery plasma corticol concentrations fall almost to zero. For extended therapeutic relief, a polymeric implants incorporating a suitable quantity of a botulinum toxin type A can be placed at the target tissue site. The implant can comprise

10   a neurotoxin, such as a of botulinum toxin type A, incorporated within biodegradable polymeric microspheres or a biodegradable pellet, either implant format containing about 10 total units (about .5 ng) of the toxin with implant characteristics of continuous release over a period of at least about four years of a therapeutic level of the toxin at point of the

15   implant release site and for a radius of about 2-3 mm on each side o the target site. The implant can release about 0.5 unit of toxin essentially immediately and further amounts of about 0.5 unit cumulatively over subsequent 2-4 months periods. The same protocol set forth in this example can be used to treat gigantism with the same etiology in

20   adolescents.

Similarly, as an alternative to use of a botulinum toxin type A, from about 5 to about 50 units of a botulinum toxin type B preparation, or from about 0.1 units to about 100 units of a botulinum toxin type C_1, D,

25   E, F or G can be stereotactically administered for extended therapeutic relief, one or more polymeric implants incorporating a suitable quantity of a botulinum toxin type B, C_1, D, E, F or G can be placed at the target tissue site.

30

Example 5
Inhibition of Gametogenesis With Botulinum Toxin Type A

To inhibit ovulation in females or sperm production in males, using CAT scan or MRI assisted stereotaxis, as set forth in Example 1 above,

5   0.5 units of a botulinum toxin type A, (such as BOTOX® or about 2 units of Dysport® or about 10 units of a botulinum toxin type B) can be is injected into the hypothalamic nucleus responsible for GnRH production. Alternately, transphenoidal surgery to locally administer the botulinum toxin to the anterior pituitary can be carried out to downregulate LH and

10   FSH production. Similarly, a polymeric implant incorporating a suitable quantity of a botulinum toxin type A can be placed at the selected target tissue site. The implant can comprise a neurotoxin, such as a of botulinum toxin type A, incorporated within biodegradable polymeric microspheres or a biodegradable pellet, either implant format containing

15   about 10 total units (about 0.5 ng) of the toxin with implant characteristics of continuous release over a period of at least about four years of a therapeutic level of the toxin at point of the implant release site and for a radius of about 2-3 mm on each side o the target site. The implant can release about 0.5 unit of toxin essentially immediately and

20   further amounts of about 0.5 unit cumulatively over subsequent 2-4 months periods. By this protocol an effective contraceptive method for use in adult males and females is provided.

Example 6
Inhibition of Menstruation With A Botulinum Toxin

25   To inhibit menstruation, CAT scan or MRI assisted stereotaxis is used, as set forth in Example 1 above, and 0.5 units of a botulinum toxin type A, (such as BOTOX® or about 2 units of Dysport® or about 10 units of a botulinum toxin type B) is injected into the area of the hypothalamic

30   responsible for inhibition of prolactin release from the pituitary, thereby, thereby inducing hyperprolactinemia. Amenorrhea results. Alternately, a polymeric implant incorporating a suitable quantity of a botulinum toxin

can be placed at the selected hypothalamic target site, as set forth in Example 5. By this protocol an effective contraceptive method for use in females is provided, as an alternate to the contraceptive method of Example 5 above where ovulation is inhibited by direct inhibition of

5   either or both GnRH and LH and FSH secretion.

Intracranial neurotoxin injection or implantation of a controlled release neurotoxin implant according to the methods of the present

10   invention can be a safe and effective therapy for patients suffering from various endocrine disorders, such as acromegaly, or to achieve contraception. Thus, a method according to the present invention can be used to treat diverse endocrine disorders, including acromegaly, Cushings disease and hyperthyroidism as well as providing effective

15   contraceptive methods.

An intracranial neurotoxin administration method for treating an endocrine disorder according to the invention disclosed herein for has many benefits and advantages, including the following:

20

1. the symptoms of an endocrine disorder can be dramatically reduced.

2. the symptoms of an endocrine disorder can be reduced for from

25   about two to about four months per injection of neurotoxin and for from about one year to about five years upon implantation use of a controlled release neurotoxin implant.

3. the injected or implanted neurotoxin exerts an intracranial target

30   tissue site specific hypothalamic releasing factor and/or pituitary hormone secretion suppressant effect.

4. the injected or implanted neurotoxin shows little or no tendency to diffuse or to be transported away from the intracranial injection or implantation site.

5   5. few or no significant undesirable side effects occur from intracranial injection or implantation of a neurotoxin according to the present invention.

6. the amount of neurotoxin injected intracranially can be
10   considerably less than the amount of the same neurotoxin required by other routes of administration (i.e. intramuscular, intrasphincter, oral or parenteral) to achieve a comparable gonadotrophin suppressant effect.

7. the gonadotrophin suppressant effects of the present methods
15   can result in the desirable side effects of greater patient mobility, a more positive attitude, and an improved quality of life.

8. effective therapeutic doses of a neurotoxin can be delivered to an intracranial target tissue over a prolonged period of time without
20   systemic toxicity.

Although the present invention has been described in detail with regard to certain preferred methods, other embodiments, versions, and modifications within the scope of the present invention are possible. For
25   example, a wide variety of neurotoxins can be effectively used in the methods of the present invention. Additionally, the present invention includes intracranial administration methods wherein two or more neurotoxins, such as two or more botulinum toxins, are administered concurrently or consecutively. For example, botulinum toxin type A can
30   be administered intracranially until a loss of clinical response or neutralizing antibodies develop, followed by administration of botulinum toxin type B. Alternately, a combination of any two or more of the

botulinum serotypes A-G can be intracranially administered to control the onset and duration of the desired therapeutic result. Furthermore, non-neurotoxin compounds can be intracranially administered prior to, concurrently with or subsequent to administration of the neurotoxin to

5   proved adjunct effect such as enhanced or a more rapid onset of hypothalamic releasing factor or pituitary hormone secretion suppression before the neurotoxin, such as a botulinum toxin, begins to exert its more long lasting suppressant effect.

10   My invention also includes within its scope the use of a neurotoxin, such as a botulinum toxin, in the preparation of a medicament for the treatment of an endocrine disorder, by intracranial administration of the neurotoxin.

15   All references, articles, patents, applications and publications set forth above are incorporated herein by reference in their entireties.

       Accordingly, the spirit and scope of the following claims should not be limited to the descriptions of the preferred embodiments set forth
20   above.